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(54) Title: NON-NATURAL GALANIN RECEPTOR LIGANDS

(57) Abstract: Compounds which are non-natural galanin receptor ligands are disclosed. The ligands are of small size, have agonist or antagonist galanin activity and may cross the blood-brain barrier to displace galanin receptors. The ligands are useful as medicaments for treatment of convulsions (e.g. in epilepsy), diseases and disorders related to endocrinology (e.g., growth hormone, insulin or prolactin release), tumors expressing galanin receptors, feeding disorders pain, allodynia, psychiatric disorders such as depression (involving e.g., noradrenaline or serotonin), cognitive disorders (e.g. Alzheimer's disease), and the like.

NON-NATURAL GALANIN RECEPTOR LIGANDS

The present invention relates to non-natural galanin receptor ligands which can function as agonists and antagonists of galanin and can be used to treat diseases or conditions in which galanin plays a role.

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BACKGROUND OF THE INVENTION

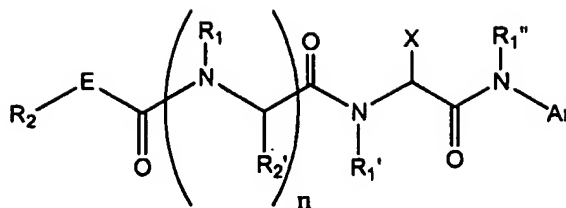
Galanin¹ is a 29/30 amino acid long neuroendocrine peptide that potently affects seizure activity, cognition, mood, feeding, and pain threshold²⁻⁶. Galanin-overexpressing mice have increased resistance to status epilepticus, while galanin knockout mice have lowered seizure threshold⁶. These results indicate that endogenous galanin is an important determinant of hippocampal excitability and of seizure threshold. The importance of galanin agonists for seizure control may arise from their ability to act at both pre- and postsynaptic sites to reduce excitability, to inhibit glutamate but not GABA release⁷, and to hyperpolarize both dentate granule cells and CA1-CA3 pyramidal cells by opening K⁺-channels⁸, dampening seizure activity. Although intracerebroventricularly injected galanin blocks seizures⁵ in mice, this large peptide is unable to cross the blood-brain barrier and is rapidly degraded.

20 To date, there are only two reports on non-peptide ligands for galanin receptors, which behave as antagonists: spirocoumaranon⁹ (Sch 202596; IC₅₀ of 1.4 μ M at human GalR1) and dithiopin-1,1,4,4-tetroxide¹⁰ (IC₅₀ of 0.17 μ M at human GalR1), despite extensive random screening efforts at six large pharmaceutical companies.

25

SUMMARY OF THE INVENTION

In accordance with the present invention, there are provided low molecular weight ligands for galanin receptor(s) having the formula:



wherein:

R_1 , R_1' , and R_1'' are each independently hydrogen or lower alkyl,

R_2 and R_2' are each independently an optionally substituted hydrocarbyl moiety containing at least about four carbon atoms,

E is optional, and, if present, is O, N or S,

X is a hydrocarbyl moiety bearing at least one substituent, wherein at least one of said substituents bears a positive charge,

Ar is an optionally substituted aromatic or heteroaromatic moiety having at least about three carbon atoms, and

n is 1, 2 or 3.

A preferred embodiment of the above compounds has been given the name "galnon": Fmoc-Cha-Lys-amidomethyl coumarin (according to IUPAC systematic nomenclature: 1-[5-amino-1-(4-methyl-2-oxo-2H-chromen-7-ylcarbamoyl)-pentylcarbamoyl]-2-cyclohexyl-ethyl-carbamic acid 9H-fluoren-9-ylmethyl ester).

The galanin receptor ligands of the invention are characterized in having galanin agonist or antagonist activity, are generally 700 daltons or less in molecular weight, and have the ability to cross the blood-brain barrier.

Also provided herein are various uses for the invention ligands, including in

vitro and in vivo analysis of galanin receptor structure and function, and use in treating a variety of diseases and conditions in which galanin plays a role. Such diseases or conditions include convulsions such as in epilepsy, growth, diabetes, pain, allodynia psychiatric states, cognition, and feeding. For such uses, the invention compounds can be formulated with an appropriate excipient(s) for pharmaceutical administration to a mammalian patient.

These and other aspects of the invention will be addressed in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a scheme for synthesis of galnon.

Figure 2. Effects of galnon and/or M35 on PTZ-induced seizures on mice. Data are presented as mean \pm SEM. Inserts show maximal seizure scores (mean \pm SEM).

a: Effect of galnon on PTZ-induced seizures. Galanin (○), galnon (▲) treated animals; and controls (◆). Asterisks indicate $p < 0.01$ versus control.

b: Effects of co-administration of M35 and galnon on PTZ-induced seizures. Animals treated with M35 (■), with M35 and galnon (□); controls (◆). Asterisks indicate $p < 0.05$ versus control.

Figure 3. Effects of galnon on self sustaining status epilepticus ("SSSE") induced by 30 min. of PPS. Data are presented as mean \pm SEM. Inserts show maximal seizure scores (mean \pm SEM).

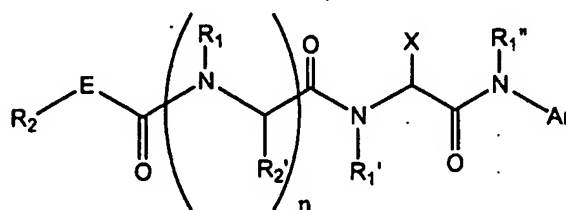
a: Intrahippocampal injection of galnon 10 min. after the end of PPS attenuated SSSE in a dose dependent manner.

b. In a model of SSSE in rats, anticonvulsant effects of both galanin and galnon were attenuated by GalR antagonist M35.

5 c. Injection of M35 (5 nM) into the hippocampus facilitated the development of SSSE. This effect was attenuated by co-administration with galnon.

DESCRIPTION OF THE INVENTION

10 In accordance with the present invention, there are provided low molecular weight ligands for galanin receptor(s) having the formula:



15

wherein:

R_1 , R_1' , and R_1'' are each independently hydrogen or lower alkyl,

20 R_2 and R_2' are each independently an optionally substituted hydrocarbyl moiety containing at least about four carbon atoms,

E is optional, and, if present, is O, N or S,

X is a hydrocarbyl moiety bearing at least one substituent, wherein at least one of said substituents bears a positive charge,

25 Ar is an optionally substituted aromatic or heteroaromatic moiety having at least about three carbon atoms, and

n is 1, 2 or 3.

It is understood that all stereoisomers of the above-identified structure are contemplated for use in the practice of the present invention.

5 As employed herein, "hydrocarbyl" refers to alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl.

 As employed herein, "alkyl" refers to hydrocarbyl radicals having 1 up to
10 about 20 carbon atoms, preferably 2-10 carbon atoms; and "substituted alkyl" comprises alkyl groups further bearing one or more substituents in place of a hydrogen atom selected from hydroxy, alkoxy, mercapto, cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, aryloxy, substituted aryloxy, halogen, oxo, cyano, nitro, amino, amido,
15 C(O)H, acyl, oxyacyl, carboxyl, carbamate, sulfonyl, sulfonamide, or sulfuryl.

 As employed herein, "lower alkyl" refers to hydrocarbyl radicals having 1 up to 6 carbon atoms.

20 As employed herein, "cycloalkyl" refers to cyclic ring-containing groups containing in the range of 3 up to about 10 carbon atoms, and "substituted cycloalkyl" refers to cycloalkyl groups further bearing one or more substituents as set forth above. Cycloalkyl groups as defined herein also refer to bicyclo- or tricycloalkyl groups, such as, for example, 2.2.1.-bicycloheptane, or adamantane.

25

 As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of 2 up

to about 12 carbon atoms, and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "cycloalkenyl" refers to cyclic ring-containing groups
5 containing in the range of 3 up to about 10 carbon atoms, wherein the cyclic ring-containing group contains at least one carbon-carbon double bond. "Substituted cycloalkenyl" refers to cycloalkenyl groups further bearing one or more substituents as set forth above.

10 As employed herein, "alkynyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of 2 up to about 12 carbon atoms, and "substituted alkynyl" refers to alkynyl groups further bearing one or more substituents as set forth above.

15 As employed herein, "aromatic" is a cyclic organic molecule which contains $4n+2$ π electrons where $n=1,2,3$ etc. Aromatic molecules are typically stable and are depicted as a planar ring of atoms with resonance structures that consist of alternating double and single bonds, e. g. benzene.

20 As employed herein, "heteroaromatic" refers to aromatic groups containing one or more heteroatoms (e.g., N, O, S,) as part of the ring structure, and having in the range of 3 up to 10 carbon atoms, optionally bearing one or more substituents as set forth above.

25 As employed herein, "heterocyclic" refers to cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S) as part of the ring structure, and having in the range of 3 up to 10 carbon atoms and "substituted heterocyclic"

refers to heterocyclic groups further bearing one or more substituents as set forth above.

It should be understood from the above that a substituted alkyl, substituted
5 cycloalkyl, substituted alkenyl, substituted alkynyl, or substituted aryl radicals may
comprise as the substituent, cycloalkyl, alkenyl, alkynyl or, alternatively, substituted
cycloalkyl, substituted alkenyl, substituted alkynyl, or substituted aryl. Thus, a
substituted form of hydrocarbyl moiety may have, as the substituent, another
substituted form of hydrocarbyl, and so forth. In general, if a substituted form of
10 hydrocarbyl is present, such hydrocarbyl is preferably not further substituted or if
substituted, the substituent comprises only a limited number of substituted
hydrocarbyl moieties, preferably 1, 2, 3, 4 or 5 such substitutions.

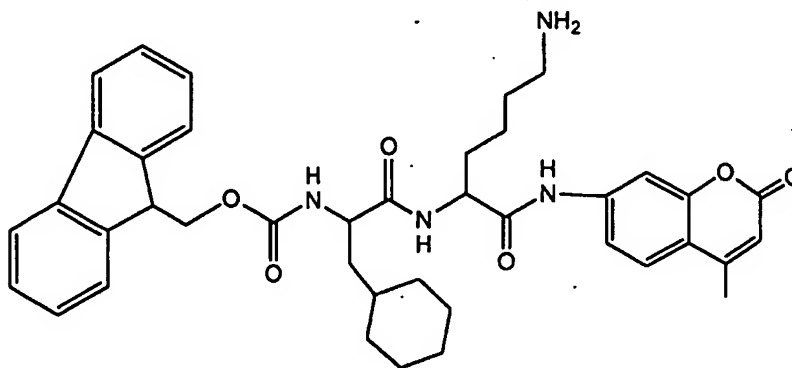
In one aspect of the invention, R_2 is a hydrophobic hydrocarbyl moiety which
15 imparts steric bulk and/or hydrophobicity to the ligand. The hydrophobic hydrocarbyl
moiety may be cyclic comprising one or more rings (e.g., mono, di, tri cyclic). Such
cyclic hydrocarbyl moiety may be further substituted as described above (e.g., alkyl,
alkenyl, or alkynyl moiety). In some cases, the further substituent (e.g., a methylene
group) may be a link between the ring of R_2 and E. The cyclic hydrocarbyl moiety
20 may be cycloalkyl, heterocyclic, aromatic, or heteroaromatic with mixtures of ring
types possible when two more rings are involved. The hydrophobic hydrocarbyl
moiety also may be a branched hydrocarbon. As employed herein, a branched
hydrocarbon has at least one hydrocarbon branch point, which is a carbon atom
attached to at least three carbon atoms. A tertiary butyl group is an example of
25 branched chain hydrocarbyl moiety. The carbon atoms after the branch points also
can be branched. R_2 also may be a combination of cyclic hydrocarbon and branched
hydrocarbon. In a preferred embodiment, R_2 comprises fluorenyl, adamantyl, or
phenyl. Most preferably R_2 comprises fluorenyl.

In another aspect of the invention, R_2' is a hydrophobic hydrocarbyl moiety which may or may not impart steric bulk to the ligand. In order to impart a degree of hydrophobicity to the ligand, R_2' typically contains at least about six carbon atoms. In one embodiment, R_2' is an optionally substituted straight or branched chain hydrocarbyl moiety. In an alternative embodiment, R_2' is an optionally substituted cyclic moiety, such as, for example, cycloalkyl, heterocyclic (e.g., pyridone), aromatic, or heteroaromatic. If a cyclic moiety is involved, it is preferably a single ring, however, R_2' may comprise multiple rings such as cycloalkyl, heterocyclic, aromatic, or heteroaromatic or combinations thereof. Such cyclic hydrocarbyl moiety may further comprise an alkyl, alkenyl, or alkynyl moiety. In some cases, the further substituent (e.g., a methylene group) may link the ring of R_2' to the compound backbone. Preferably, R_2' comprises cyclohexylmethyl or benzyl. In addition, those skilled in the art will recognize that R_2' may be incorporated into invention compounds as a side chain of a hydrophobic amino acid, such as, e.g., tyrosine, tryptophan or dihydroxyphenyl phenylalanine. The side chain may be in the D or L configuration.

In a further aspect of the invention, X is a straight or branched chain alkyl, containing at least one substituent bearing a positive charge. Those skilled in the art recognize that there are a variety of well-known substituents with the ability to bear, under appropriate conditions (e.g., low pH) a positive charge, such as, for example, hydroxy groups, amines, or thiols which when positively charged are referred to as hydronium, ammonium and sulfonium, respectively. In addition, those skilled in the art recognize that X may be incorporated into invention compounds as a side chain of an amino acid, such as, e.g., lysine, arginine, histidine, asparagine, or glutamine. In a most preferred embodiment, the side chain is from lysine. The side chain may be in the D or L configuration.

In yet another aspect of the invention, Ar is an optionally substituted aromatic or heteroaromatic moiety having at least about three carbon atoms. In one embodiment, Ar comprises an optionally substituted aromatic, such as, for example, phenyl or naphthyl. In an alternative embodiment, Ar comprises an optionally substituted heteroaromatic. Preferably, the heteroaromatic comprises chromenyl, furyl, thiophenyl, oxadiazolyl, benzoxazolyl, benzofuryl, benzodioxolyl, imidazolyl, benzimidazolyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, isoxazolyl, triazolyl, benzotriazolyl, indolyl, pyrimidinyl, pyrazolyl, quinoliny, isoquinoliny, quinazoliny, or pyridyl. Most preferably, Ar comprises chromenyl.

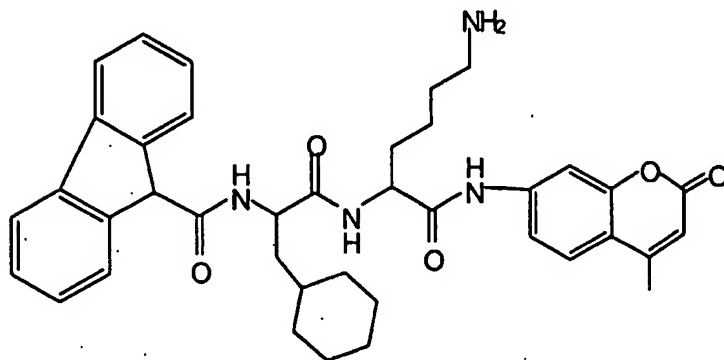
A particular compound suitable for use in the practice of the present invention has been given the name "galnon": Fmoc-Cha-Lys-amidomethyl coumarin (according to IUPAC systematic nomenclature: 1-[5-amino-1-(4-methyl-2-oxo-2H-chromen-7-ylcarbamoyl)-pentylcarbamoyl]-2-cyclohexyl-ethyl-carbamic acid 9H-fluoren-9-ylmethyl ester). This agonist of the invention inhibits adenylate cyclase activity in vitro, and attenuates pentylenetetrazole-induced seizures in vivo when administered systemically. These findings introduce and support the efficacy of a new class of antiepileptic agents, which are effective in inhibiting a variety of seizure types. This particular galanin receptor agonist has the structure:



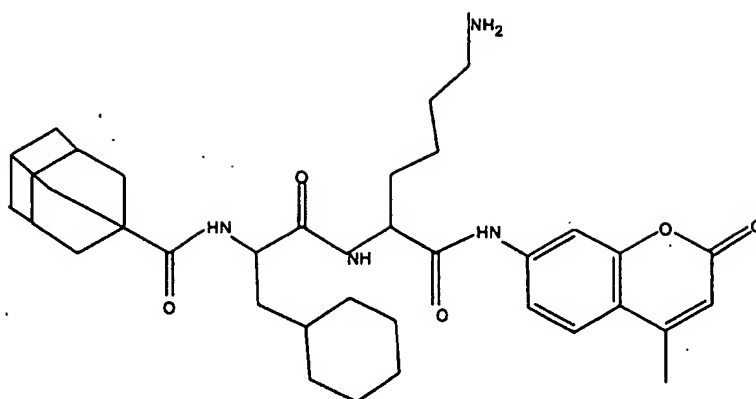
The above compound from left to right is composed of a bulky hydrophobic group (i.e., the Fmoc group) coupled to a hydrophobic amino acid residue, which is coupled to an amino acid residue with a protonated side-chain, which is finally coupled to an aromatic amine. In addition, this compound has the ability to cross the blood-brain barrier and to displace galanin from galanin receptors.

In an alternative embodiment, the bulky hydrophobic group is derived from fluorenylcarboxylic acid, as depicted in the exemplary structure shown below:

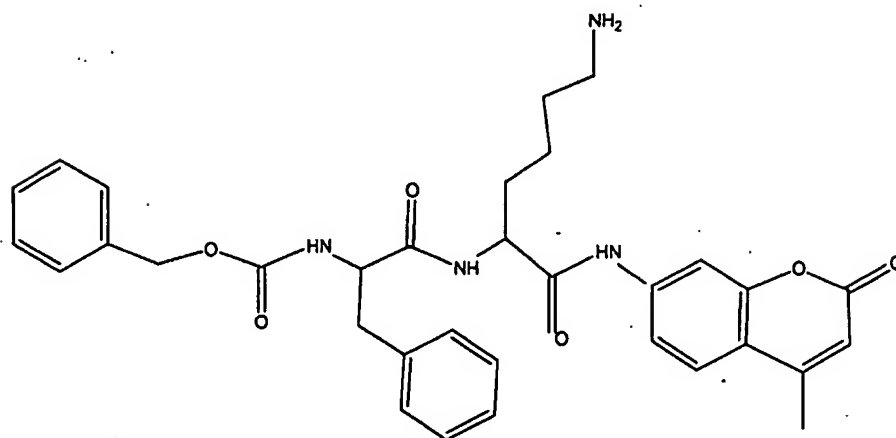
10



In an embodiment of the invention a galanin receptor ligand L-138 has the formula:



- 5 In another embodiment of the invention a galanin receptor ligand L-131 has the formula:



10

It should be understood that the cyclohexylalanine group of the compound L138 and galnon, the phenylalanine group of compound L131, and the lysine group of compounds L131, L138 and galnon may be in D-, L- or DL- form.

Galanin receptor ligands of the invention non-natural compounds that have the ability to displace galanin from its receptor. A "receptor ligand" as used herein with respect to galanin is a non-natural compound generally small in size, having a molecular weight of 10,000 daltons (Da) or less, preferably 1,000 Da or less and more preferably
5 700 Da or less. Galanin receptor ligands bind to the galanin receptor and displace natural galanin, exhibiting an affinity for the receptor in the micromolar range or lower (e.g. nanomolar).

Galanin receptor ligands of the invention have agonist or antagonist activity.
10 As employed herein, a galanin receptor ligand has agonist activity with respect to the galanin receptor if it activates the galanin receptor in a manner that is similar to that achieved with native galanin. A galanin receptor ligand with antagonist activity blocks or inhibits activation of the galanin receptor by native galanin. The galanin receptor ligands including galnon, L131 and L138 are galanin agonists.

15 Galanin receptor ligands of the invention preferably have the property of being able to cross the blood brain barrier. As employed herein, the blood brain barrier refers to the endothelial cell lining of blood vessels located in the central nervous system. Galanin receptor ligands can cross the blood brain barrier following
20 administration into blood by virtue of their small size (generally about 700 Da or less) and hydrophobic character. Alternatively, the invention compounds may cross the blood brain barrier by coupling them to a peptide naturally transfers by receptor-mediated peptide transport (e.g. insulin).

25 Galanin receptor ligands of the invention have utility for dissecting the mechanism of galanin's action. For example, galanin receptor ligands with selective antagonistic effects can be used to determine structural features of the galanin receptor that plays a role in the complex biological effects attributed to the receptor. Galanin

receptor ligands also can be used in structure function analysis to identify key moieties or substituents that impart useful features for drug delivery in vivo, an analysis that can be conducted in an animal model.

5 There are various clinical uses for galanin receptor ligands of the invention. Galanin receptor ligands which have agonist activity can be used for treating various disorders or conditions. In the case of CNS, agonist ligands can be used to treat convulsive seizures such as arise in the case of epilepsy. Such invention compounds also may be used for CNS injuries or in open hear surgery to prevent anoxic damage.

10 Galanin receptor ligand agonists also are useful in neuroendocrinology disorders. For example, dwarfism can be treated by administering a galanin agonist ligand which acts to increases release of growth hormone. Galanin receptor ligand agonists also can be used to treat endocrinology disorders that arise as a result of cancers that express galanin receptors. For example, the invention galanin receptor agonists can be used to

15 control prolactin or insulin release from pituitary adenomas. Galanin receptor ligand agonists may be used as an analgesic for pain control. Invention compounds can be used, for example, to control the pain threshold or prolong the effect of other analgesics such as morphine. This may allow lower doses of morphine, which is particularly useful for treating chronic pain.

20

 Galanin receptor ligands of the invention which have antagonist activity can be used for treating various disorders or conditions. Invention ligand antagonists can be used to improve cognitive function in Alzheimer's disease by improving cholinergic function negatively impacted by galanin. Invention ligand antagonists also may be

25 used as antidepressants by, for example, enhancing firing of noradrenergic neurons or by suppressing 5-HT metabolism. Galanin receptor ligand antagonists can be used to treat feeding disorders or obesity by suppressing fat or carbohydrate intake.

In accordance with these uses, galanin receptor ligands can be formulated for pharmaceutical use by combining the receptor ligand with a pharmaceutically acceptable excipient(s). The excipients are selected by the manufacturer depending on the desired form of the preparation, e.g. tablets and solutions or suspensions for
5 injection, and the desired route of administration, and examples of suitable excipients can be found in the US or European pharmacopoeia. In a presently preferred embodiment of the pharmaceutical preparation according to the invention, the preparation is in the form of an intraperitoneal injection solution.

10 Another aspect of the invention is to use the galanin receptor ligands as a medicament. Further, the medicament may be selected from the group consisting of medicaments for the treatment of diseases and disorders as described above including growth hormone release, insulin release, tumors expressing galanin receptors, pain states such as allodynia, psychiatric states such depression (e.g., where noradrenaline
15 or serotonin activity need be modulated), and the like.

The galanin receptor ligands of the invention can be used prophylactically or therapeutically to treat the diseases or disorders discussed above by administering a prophylactically or therapeutically effective dose of a galanin receptor agonist
20 according to the invention or a pharmaceutical preparation according to the invention, to the patient. The mammalian patient may be a human patient. The prophylactically or therapeutically effective anticonvulsant dose of a galanin receptor agonist or a pharmaceutical preparation of the invention will be recommended by the attending physician with guidance from the manufacturer and the response from the patient.
25 Thus, the invention compounds may be administered in an effective dose to a patient prophylactically or therapeutically to treat a disease or disorder related to seizures, growth hormone release, insulin release deviations, tumors expressing galanin receptors, pain states, allodynia, and noradrenaline or serotonin activity, and the like in

mammalian patient.

The invention will now be illustrated with experiments and accompanying drawings, but it should be understood that the invention is not limited to any specifically mentioned details.

EXAMPLES

Abbreviations commonly used in the field of chemistry and biology are used in this specification. In particular, the following abbreviations are used: AMC, 7-amino-4-methylcoumarin; Boc, *tert*-butoxycarbonyl; Cha, cyclohexylalanine; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; i.p., intraperitoneally; PTZ, pentylenetetrazole; TBTU, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid.

A. Synthesis of galanin receptor agonists of the invention

The synthesis strategy is shown in Fig. 1 for the synthesis of galnon, and the synthesis of the compounds L138 and L131 followed essentially the same strategy.

The first step of the galnon synthesis was the coupling of Fmoc-Lys(Boc)-OH to AMC. 1 mmol of Fmoc-Lys(Boc)-OH and 0.5 equivalents of DCC were separately dissolved in dioxane, cooled on ice and then pooled. Reaction mixture was stirred for 30 min at room temperature, then 0.5 equivalents of AMC dissolved in DMF was added to the symmetric anhydride solution and the mixture was stirred overnight. Next day the solvents were evaporated, Fmoc-Lys(Boc)-AMC was precipitated with petrol ether/ ethyl acetate mixture (5/95 v/v) and dried under vacuum. The Boc-group

was removed with H₂O/TFA mixture (3/97 v/v) in ice bath for 5 mm, followed by the evaporation of the solvents. The obtained Fmoc-Lys-AMC was coupled to chlorotriyl resin by incubation of 2-3 equivalents of Fmoc-Lys-AMC, 4-9 equivalents of DIEA and 1 equivalent of chlorotriyl resin for 2 h. Resin was washed and the Fmoc-group
5 was removed with piperidine/DMF (20/80 v/v). Coupling of Fmoc-Cha was performed using 2 equivalents of amino acid, TBTU, HOBt and 4 equivalents of DIEA.

Galnon was cleaved from the resin by applying TFA/DCM mixture (50/50 v/v)
10 in four aliquots. The filtrate was evaporated and the obtained product was purified on Sep-Pak® Cartridges and analyzed using plasma desorption mass spectrometer (model Bioion 20, Bioion, Uppsala, Sweden).

15 B. Characterization of the biological properties of the agonists of the invention

First, human and rat galanin were synthesized on the model 431A peptide synthesizer (Perkin Elmer Applied Biosystems Inc., Foster City, California).

20 1. Ligand binding to galanin receptors, adenylate cyclase assay

The mixtures of ligands and individual compounds were screened to displace [¹²⁵I]-galanin in equilibrium binding assay using membrane preparations from rat hippocampi, rich in galanin binding sites as described in detail previously¹¹. Effect of galnon on basal and forskolin-stimulated cAMP production were performed
25 essentially as described by Valkna et al.¹² in membranes from rat ventral hippocampi.

2. Autoradiography

Rats were decapitated, the spinal cord lumbar segments L4 and L5 were

dissected out, rapidly frozen, cut in a cryostat and processed for the autoradiographic ligand binding assay of Young and Kuhar¹³, as described previously¹⁴. Galnon was added at 1, 3 and 5 μ M concentration. The sections were processed in a BAS3000 Bio-Imagine Analyzer (Fuji) and quantified and then exposed to Hyperfilm-Max X-ray film (Amersham Pharmacia Biotech).

3. Seizure induction and quantification

The experiments were performed on C57B1 male mice (weight 20-30 g).
10 Animals to be treated with galanin, or M35 were anaesthetized with ketamine (100 mg/kg i.p.) and xylazine (15 mg/kg i.p.) and stereotactically implanted i.c.v. with guide cannulae (0.6 mm internal diameter). Postoperative recovery was 3-5 days. Galanin, or M3 5 were injected into the lateral brain ventricle of freely moving mice by means of Hamilton microsyringe in the amount 0.5 nmol, in 0.5 μ l, at a rate 0.5 min/min.
15 Control animals were treated with saline.

Galnon was dissolved in the 50% DMSO in saline and administered i.p. in a dose of 2 mg/kg 15 mm prior to PTZ injection (when the effect of galnon alone was studied) or 5 mm after M35 injection (in co-administration studies). Control animals
20 were treated with 50% DM50 in saline.

Seizures were induced by i.p. injection of PTZ (Research Biochemicals, Natick, Massachusetts) in a dose of 40 mg/kg (when studying the effects of galanin only, or galnon only) or 30 mg/kg (when studying the effect of M35 and galnon +
25 M35). The animals were placed in individual Plexiglas cages and videotaped for 15 min after PTZ administration. Seizures were analyzed off-line by a unbiased investigator and quantified using the following scale: 0: no motor seizures; 1: staring,

mouth, or facial movements; 2: head nodding or isolated twitches; 3: unilateral
bilateral forelimb clonus; 4: rearing; 5: rearing and falling; 6: tonic extension of hind
limbs or death. The latency of each behavioral seizure type and the highest seizure
score were recorded. For statistical purposes, if the animal failed to show seizure of
5 any particular score, the latency of 900 s (which was a period of observation) was
assigned to this score. No obvious behavioral side effects of galnon were observed.
Statistical methods used: t-test (latency) and Mann-Whitney (seizure score).

C. Summary of results

10

The synthesized compound galnon was tested for the ability to displace [125 I]-
galanin from galanin receptors in rat ventral hippocampal membranes. [125 I]-Galanin
was displaced by galnon in membranes from rat ventral hippocampus with a K_i value
4.8 μ M (Table 1).

15

The ability of galnon to displace [125 I]-galanin from its binding sites in a
concentration-dependent manner was confirmed in an autoradiographic binding assay
on rat spinal cord sections, where the autoradiographic signal of [125 I]-galanin and its
dose-dependent displacement by galnon could be studied best. A significant increase
20 in binding as compared to controls was only seen at the concentration of 5 μ M.

25

Like galanin, galnon inhibited both basal and forskolin- stimulated adenylate
cyclase activity (Table 1). Inhibition of adenylate cyclase activity suggested that
galnon exhibited agonist-like properties at galanin receptor(s).

To study the potential agonist properties of galnon, we tested its anticonvulsant
properties. To test anticonvulsant capability of galnon, we used pentylenetetrazol
(PTZ)-induced convulsions in mice. In the first set of experiments, we induced

seizures with 40mg/kg of PTZ (i.p.), and injected 2 mg/kg of galnon (i.p.) 15 min prior to PTZ injection. The galnon treatment lowered the maximal seizure score from 4.5 (control mice) to 1.45 (galnon-treated mice), $p < 0.01$ (Fig. 2a). The anticonvulsant effect of galnon was comparable to that of galanin administered i.c.v. in a dose 0.5 nmol.

The next set of experiments was designed to confirm that the antiepileptic activity of galnon is exerted at galanin receptors. Specifically, we co-administered M35 (i.c.v.), a galanin receptor antagonist that does not cross the blood-brain barrier, and galnon (i.p.). Seizures were then induced with 30 mg/kg PTZ. Galnon completely abolished the seizure-facilitating effect of M35 (Fig. 2b), the galanin antagonist that, as demonstrated earlier⁶, potentiates PTZ induced seizures.

The effects of intrahippocampal galnon administration was evaluated in rats. SSSE consisted of recurrent limbic seizures of varying intensity (stages 1-5), which lasted for 12-18 h after PPS was discontinued. Behavioral seizures were accompanied by high-frequency (13+ Hz) and amplitude (1+ mV) discharges with the duration of individual events between 30 s and 3 min. Between the seizures, ictal spikes with the amplitude of 0.8 mV and more and frequency of 3 Hz and less were continuously generated (spike frequency of 3 Hz and more was recognized as a seizure)⁵.

Intrahippocampal administration of galnon shortened the duration of SSSE and decreased the time spent in seizures in a dose-dependent manner (Fig. 3a). In the maximal dose used (5 nmol), galnon shortened SSSE duration to 28 ± 8 min, from 760 ± 77 min in controls ($P < 0.05$). The anticonvulsant effects of galnon were abolished by pretreatment with the galanin receptor antagonist M35, when the latter was administered in a dose that alone, as it had been previously reported⁵, did not alter the course of SSSE (0.5 nmol, Fig. 3b). When galnon was injected immediately after

M35, both time spent in seizures and the duration of SSSE were significantly higher than in galnon-treated rats without M35, and these parameters did not differ from those in control animals (Fig. 3b).

5 In a separate set of experiments, equimolar galnon blocked the seizure-facilitating effect of intrahippocampal injections of M35 in an amount of 5 nmol, a dose, which was previously shown to potentiate seizures⁶: under these conditions PPS as short as 7 min is sufficient to induce self-sustaining seizures⁶. When an equimolar amount of galnon was administered into the hippocampus before M35, total seizure
10 time and duration of SSSE were reduced from 45 ± 5 to 13 ± 4 min and from 99 ± 11 to 37 ± 8 min, respectively (Fig. 3c).

 We examined the anticonvulsant effects of galnon in SSSE. As shown previously, down-regulation of GalR1 by PNA1 did not affect the parameters of
15 SSSE⁵. However, in PNA1-pretreated animals, galnon (1 nmol) had no effect on self-sustaining seizures, whereas in control rats injected with scrambled PNA, galnon reduced total seizure time 9-fold and duration of SSSE 7-fold.

 In summary, the synthesis of galnon, Fmoc-Cha-Lys-amidomethylcoumarin, a
20 low molecular weight, blood brain barrier-penetrating galanin receptor ligand with agonist properties, both *in vitro* and *in vivo* is described. Galanin exerts inhibitory effects on excitatory transmission by inhibition of glutamate release in the ventral hippocampus, by hyperpolarization of granule as well as of pyramidal cells through enhanced K^+ conductance and reduced L-type Ca^{2+} channel opening. Therefore,
25 galanin receptor agonists are expected to act as very potent antiepileptic agents in seizures of different origins. The experiments herein show that a systemically active galanin receptor agonist is a potent anticonvulsant against PTZ-induced seizures in mice, and is useful in a broad spectrum of seizure and epilepsy models.

The invention thus has been disclosed broadly and illustrated in reference to representative embodiments described above. Those skilled in the art will recognize that various modifications can be made to the present invention without departing from the spirit and scope thereof. All publications, patent applications and issued
5 patents, are herein incorporated by reference to the same extent as if each individual publication, patent application or issued patent were specifically and individually indicated to be incorporated by reference in its entirety.

D. References

1. Tatemoto, K., Rökaeus, Å, Jörnvall, H., McDonald, T.J. & Mutt, V. Galanin -
5 a novel biologically active peptide from porcine intestine. *FEBS Lett.* **164**,
124-128 (1983).
2. Bartfai, T. & Langel, Ü. Galanin receptor ligands as potential therapeutic
agents in depression and neurodegeneration. *Eur. J. Med. Chem.* **30**, S163-
S174 (1995).
- 10 3. Bartfai, T. Galanin: a neuropeptide with important central nervous system
actions. in *Psychopharmacology: the Fourth Generation of Progress* (eds
Bloom, F.E. & Kupfer, D.J.) 563-571 (Raven Press, 1185 Ave of the
Americas, New York, NY 10036, 1995).
4. Langel, Ü. & Bartfai, T. Chemistry and molecular biology of galanin receptor
15 ligands. *Ann. NY Acad. Sci.* **863**, 86-93 (1998).
5. Mazarati, A. *et al* Galanin modulation of seizures and seizure modulation of
hippocampal galanin in an animal model of status epilepticus. *J. Neurosci.* **18**,
10070-10077 (1998).
6. Mazarati, A.M. *et al* Modulation of hippocampal excitability and seizures by
20 galanin. *J. Neurosci.* **20**, 6276-81 (2000).
7. Zini, S., Roisin, M.P., Langel, Ü., Bartfai, T. & Ben-An, Y. Galanin reduces
release of endogenous excitatory amino acids in the rat hippocampus. *Eur. J.*
Pharmacol -Molec. Pharm. **245**, 1-7 (1993).
8. Xu, Z.Q., Ma, X., Soomets, U., Langel, Ü. & Hökfelt, T. Electrophysiological
25 evidence for a hyperpolarizing, galanin (1-15)- selective receptor on
hippocampal CA3 pyramidal neurons. *Proc. NatL Acad. Sci. USA* **96**, 14583-7
(1999).

9. Chu, M. *et al* A new fungal metabolite, Sch 202596, with inhibitory activity in the galanin receptor GaIR1 assay. *Tetrahedron Lett.* **38**, 6111-6114 (1997).
10. Scott, M.K. *et al* 2,3-Dihydro-dithiin and -dithiepine-1,1,4,4-tetroxides: small molecule non-peptide antagonists of the human galanin hGAL-1 receptor.
5 *Bioorg. Med. Chem.* **8**, 1383-91 (2000).
11. Land, T., Langel, Ü, Fisone, G., Bedecs, K. & Bartfai, T. Assay for galanin receptor. *Methods in Neurosciences* **5**, 225-234 (1991).
12. Valkna, A. *et al*. Differential regulation of adenylate cyclase activity in rat ventral and dorsal hippocampus by rat galanin. *Neurosci Lett.* **187**, 75-78 (1995).
- 10 13. Young, W.S., 3rd & Kuhar, M.J. A new method for receptor autoradiography: [3H] opioid receptors in rat brain. *Brain Res.* **179**, 255-270. (1979).
14. Pooga, M. *et al* Cell penetrating PNA constructs regulate galanin receptor levels and modify pain transmission *in vivo*. *Nature Biotechnology* **16**, 857-861 (1998).
- 15 15. Land, T. *et al* Linear and cyclic N-terminal galanin fragments and analogs as ligands at the hypothalamic galanin receptor. *Int. J. Peptide Protein Res.* **38**, 267-272 (1991).
16. Valkna, A. *et al* Effects of chimeric galanin receptor ligands on basal adenylate cyclase activity in rat ventral hippocampal membranes. *Protein Peptide Lett.* **2**, 267-274 (1995).

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Table 1. Comparison of galanin and galnon in binding to galanin receptors and inhibiting adenylate cyclase (basal and forskolin stimulated) in membranes from rat ventral hippocampi.

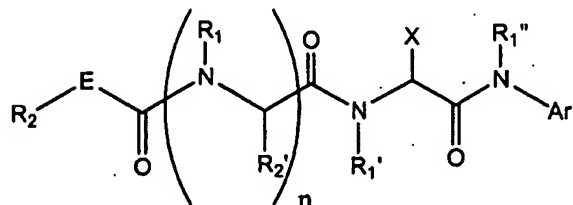
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	Galanin, rat ^{15,16}	Galnon
MW, Da	3209	677
K_i M	$0.8 \cdot 10^{-9}$	$4.8 \pm 0.6 \cdot 10^{-6}$
EC₅₀, M of inhibition of adenylate cyclase activity	$1.1 \cdot 10^{-9}$ (basal)	$8.0 \pm 3.0 \cdot 10^{-6}$ (basal)
	$1.1 \cdot 10^{-9}$ (forskolin stimulated)	$10 \pm 3 \cdot 10^{-6}$ (forskolin stimulated)

10

CLAIMS

1. A compound which is a galanin receptor ligand having the formula:



wherein:

R_1 , R_1' , and R_1'' are each independently hydrogen or lower alkyl,

R_2 and R_2' are each independently an optionally substituted hydrocarbyl moiety containing at least about four carbon atoms,

E is optional, and, if present, is O or S,

X is a hydrocarbyl moiety bearing at least one substituent, wherein at least one of said substituents bears a positive charge,

Ar is an optionally substituted aromatic or heteroaromatic moiety having at least about three carbon atoms, and

n is 1 to 3.

2. A compound according to claim 1, wherein R_2 is an optionally substituted branched chain hydrocarbyl moiety.

3. A compound according to claim 1, wherein R_2 is an optionally substituted cyclic hydrocarbyl moiety.

4. A compound according to claim 3, wherein said cyclic moiety is cycloalkyl, heterocyclic, aromatic, or heteroaromatic.
5. A compound according to claim 4, wherein said cyclic moiety is polycyclic.
- 5 6. A compound according to claim 4, wherein said cyclic moiety is aromatic.
7. A compound according to claim 1, wherein R_2' contains at least six carbon atoms.
- 10 8. A compound according to claim 7, wherein R_2' is an optionally substituted straight or branched chain hydrocarbyl moiety.
9. A compound according to claim 7, wherein R_2' is an optionally substituted cyclic moiety.
- 15 10. A compound according to claim 9, wherein said cyclic moiety is cycloalkyl, heterocyclic, aromatic, or heteroaromatic.
11. A compound according to claim 1, wherein X is a straight or branched chain alkyl.
- 20 12. A compound according to claim 11, wherein said substituent is amine, hydroxy, or thiol.
13. A compound according to claim 11, wherein said substituent is amine.
- 25 14. A compound according to claim 1, wherein said Ar is optionally substituted aromatic.

15. A compound according to claim 14, wherein said Ar is optionally substituted phenyl or naphthyl.

5 16. A compound according to claim 1, wherein said Ar is optionally substituted heteroaromatic.

17. A compound according to claim 16, wherein said Ar is chromenyl, furyl, thiophenyl, oxadiazolyl, benzoxazolyl, benzofuryl, benzodioxolyl, imidazolyl, benzimidazolyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, isoxazolyl, triazolyl, 10 benzotriazolyl, indolyl, pyrimidinyl, pyrazolyl, quinolinyl, isoquinolinyl, quinazolinyl, or pyridyl.

18. A compound according to claim 1, where R₂ and R₂' are substituted with a hydrocarbyl moiety containing at least about four carbon atoms comprising a selected from the group consisting of hydroxy, alkoxy, mercapto, cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, aryloxy, substituted aryloxy, halogen, oxo, cyano, nitro, amino, amido, C(O)H, acyl, oxyacyl, carboxyl, carbamate, sulfonyl, sulfonamide, or sulfonyl.

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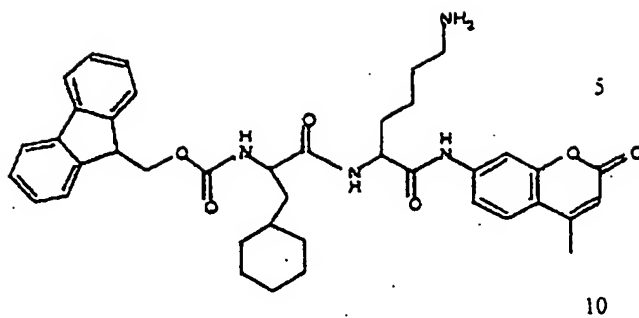
19. A compound according to claim 1, wherein n is 1.

20. A compound according to claim 1 having a molecular weight of 700 daltons or less.

25

21. A compound according to claim 1 which crosses the blood-brain barrier.

22. A compound according to claim 1 having the formula



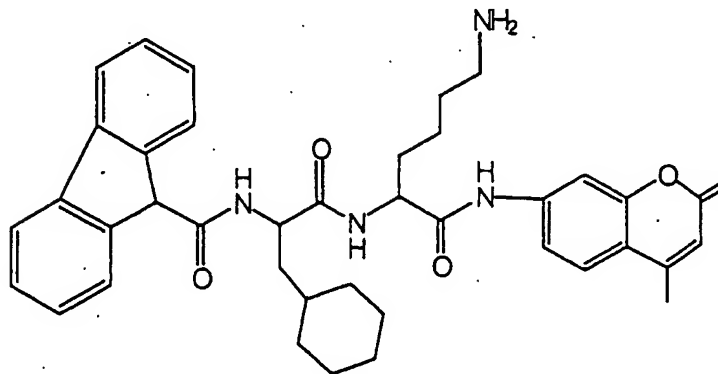
(1 -[5-amino-1-(4-methyl-2-oxo-2H-chromen-7-ylcarbamoyl)-pentylcarbamoyl]-2-cyclohexyl-ethyl-carbamic acid 9H-fluoren-9-ylmethyl ester).

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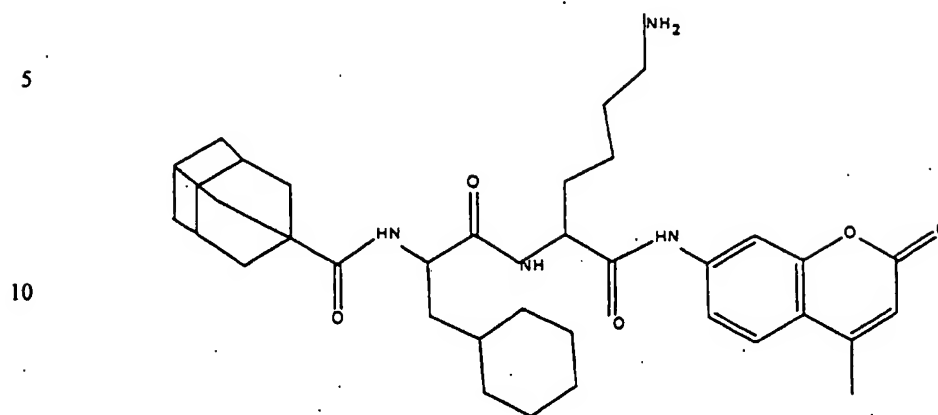
23. A compound according to claim 1 having the formula

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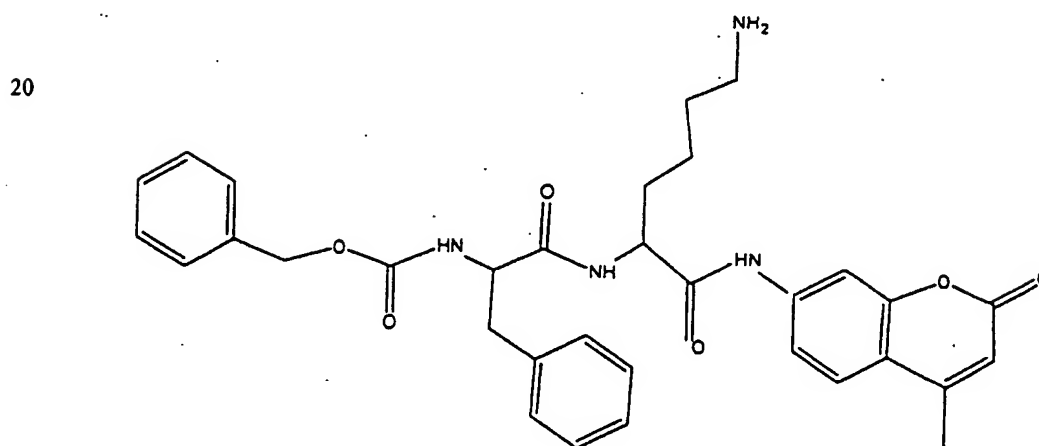


24. A compound according to claim 1 having the formula



15

25. A compound according to claim 1 having the formula



26. A pharmaceutical preparation comprising the compound according to claim 1 and a pharmaceutically acceptable excipient(s).
27. A pharmaceutical preparation according to claim 26 in the form of an
5 intraperitoneal injection solution.
28. The compound according to claim 1 for use as a medicament.
29. The compound according to claim 28, wherein the medicament is selected from
10 the group consisting of anticonvulsant and antiepileptic agents.
30. The compound according to claim 28, wherein the medicament is selected from the group consisting of medicaments for the treatment of diseases and disorders related to growth hormone release, insulin release, tumors expressing galanin
15 receptors, pain states, allodynia psychiatric states, cognition, and feeding.
31. A method of prophylactically or therapeutically treating a disease or disorder resulting in convulsive seizures in a mammalian patient, comprising administering a prophylactically or therapeutically effective anticonvulsant dose of the compound
20 according to claim 1 or a pharmaceutical preparation according to claim 22 to the patent.
32. The method according to claim 31, wherein the convulsive seizures are due to
25 epilepsy.
33. A method of prophylactically or therapeutically treating a disease or disorder related to growth hormone release, insulin release, tumors expressing galanin receptors, pain states, allodynia , psychiatric states, cognition, and feeding in a mammalian patient, comprising administering a prophylactically or therapeutically
30 effective dose of the compound according to claim 1 or a pharmaceutical preparation according to claim 26 to the patent.

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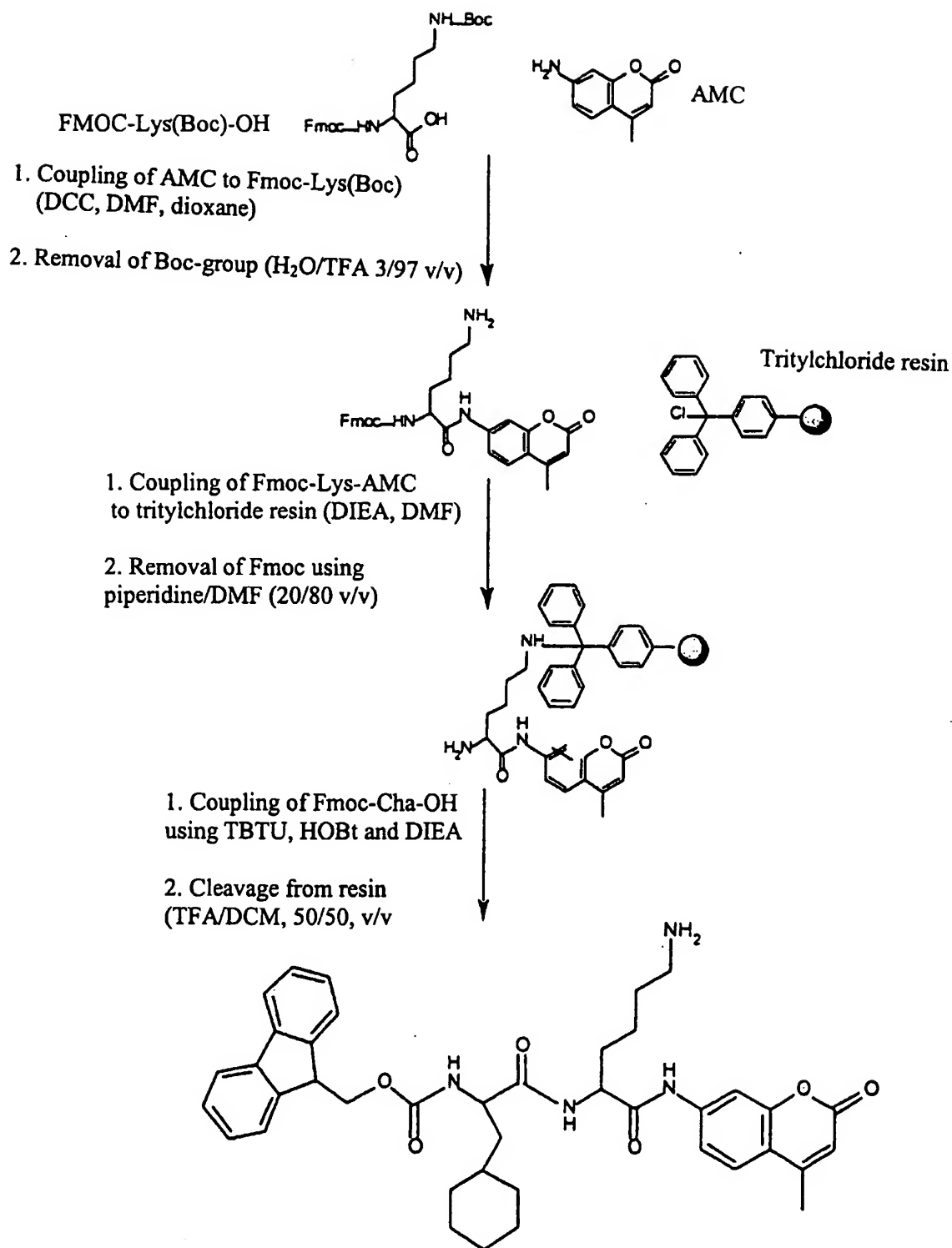


Fig. 1.

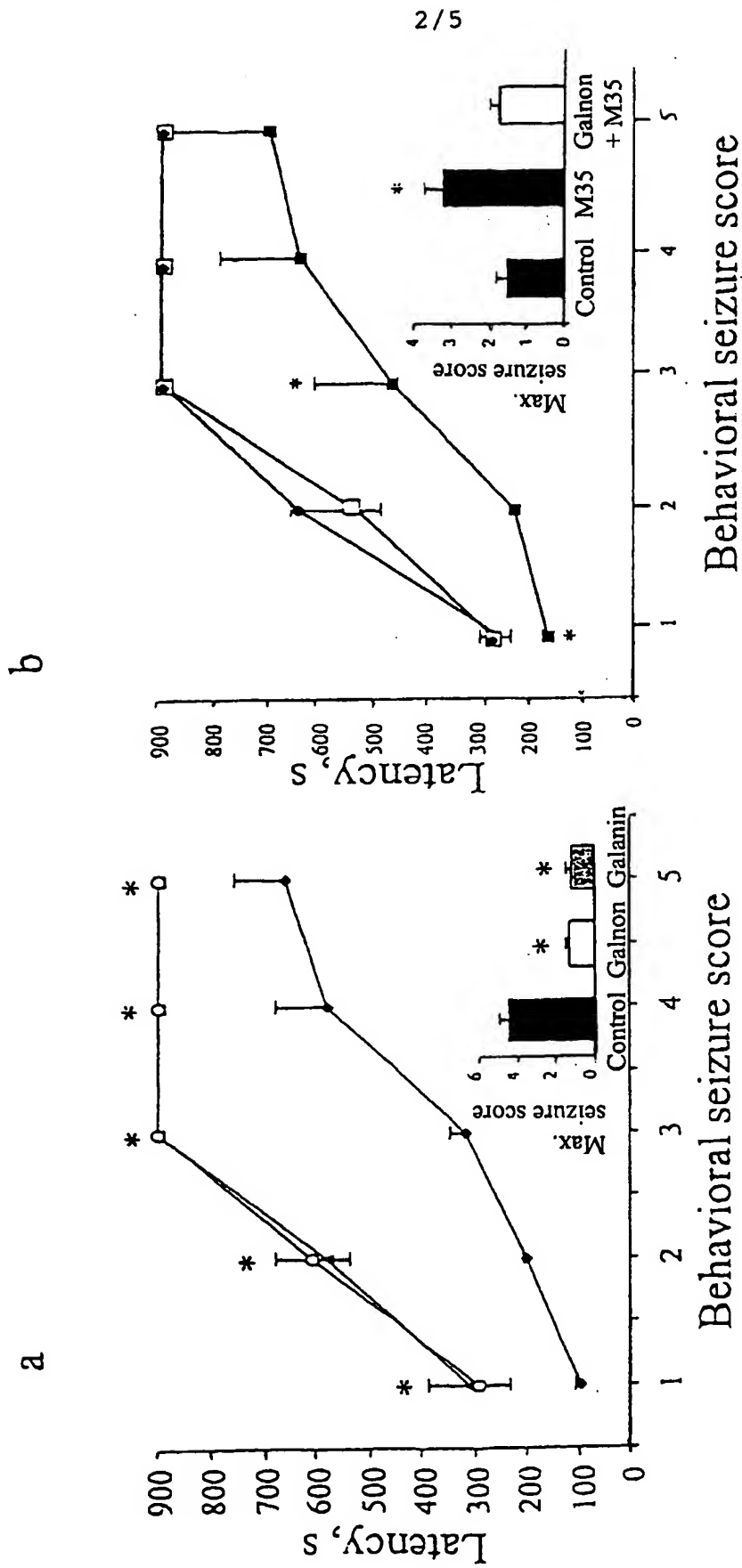
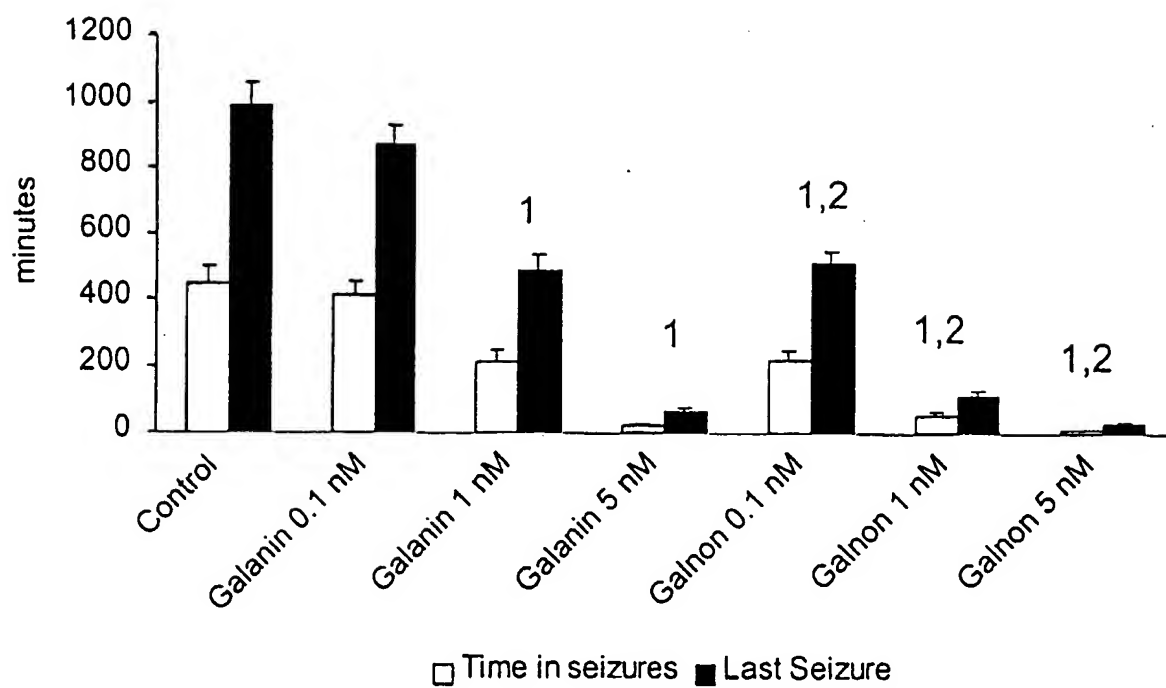


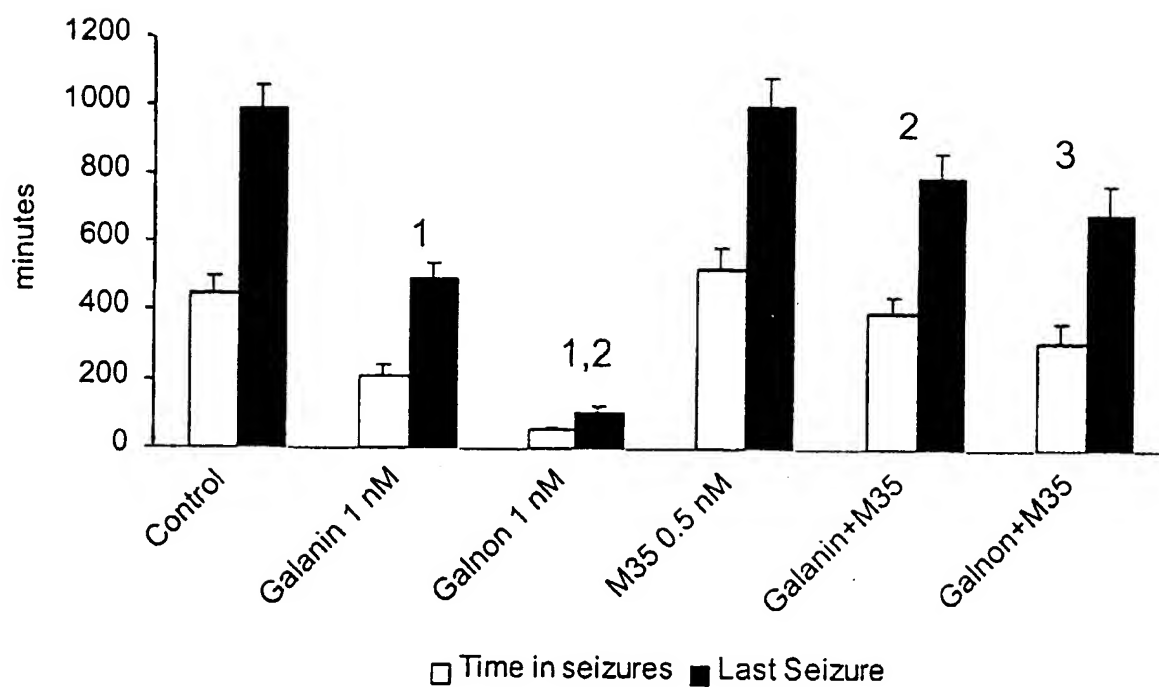
FIG. 2



$P < 0.05$: 1 vs. Control, 2 vs. equimolar amount of Galanin

Fig. 3a.

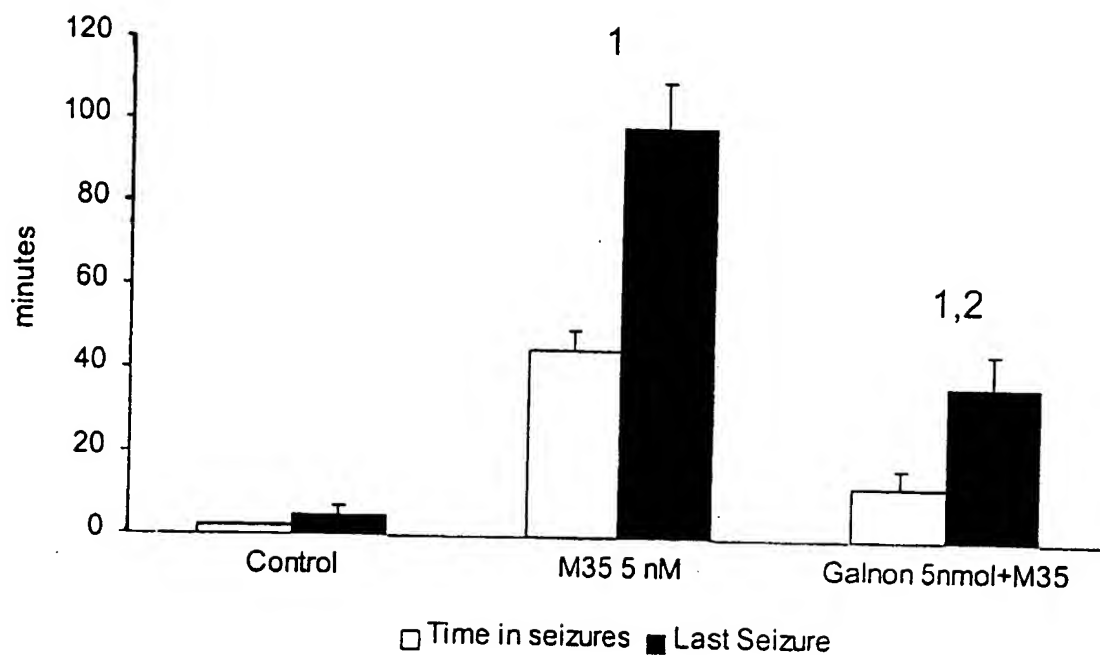
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P<0.05: 1 vs. Control, 2 vs. Galanin, 3 vs. Galnon

Fig.3b.

5/5



$P < 0.05$: 1 vs. Control, 2 vs. M35

Fig.3c.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 02/01002

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 5/06, A61K 38/05, A61P 25/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, CHEM. ABS DATA, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Protein Expression and Purification; Volume 24, 2002, Brian M. Cain et al: "Production, Purification, and Characterization of Recombinant Prohormone Convertase 5 from Baculovirus-Infected Insect Cells", page 227 - page 233 --	1-19,25
X	STN International, File ZCAPLUS, ZCAPLUS accession no. 2001:9514, Document no. 134:189890, Abe, Naoko et al: "Design and synthesis of sensitive fluorogenic substrates specific for Lys-gingipain"; Journal of Biochemistry (Tokyo) (2000), 128(5), 877-881 --	1-4,6-19

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 October 2002

Date of mailing of the international search report

21-10-2002

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 02/01002

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File ZCAPLUS, ZCAPLUS accession no. 1980:193362, Document no. 92:193362, Iwanaga, S. et al: "Fluorogenic peptide substrates for proteases in blood coagulation, kallikrein-kinin and fibrinolysis systems"; & Adv. Exp. Med. Biol. (1979), Volume Date 1978, 120A(Kinins 2: Biochem., Pathophysiol., Clin. Aspects), 147-63 --	1,7-8,11-19
A	Neuropeptides, Volume 34, no. 6, 2000, Anders Florén et al: "Galanin receptor subtypes and ligand binding", page 331 - page 337 -- -----	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE02/01002

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **31-33**
because they relate to subject matter not required to be searched by this Authority, namely:
see next sheet
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE02/01002

Claims 31-33 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.